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Letter to the Editor: Solution structure of chicken liver basic fatty acid binding protein

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Abstract

Chicken liver basic fatty acid binding protein (Lb-FABP) belongs to the basic-type fatty acid binding proteins, a novel group of proteins isolated from liver of different non mammalian species whose structure is not known. The structure of Lb-FABP has been solved by ^{1}H NMR. The overall fold of Lb-FABP, common to the other proteins of the family, consists of ten antiparallel β-strands organised in two nearly ortogonal β-sheets with two alpha helices closing the protein cavity where small hydrophobic ligands can be bound. The binding specificity of the protein is not known, however, based on the high sequence and structural similarity with an orthologous protein, ileal lipid binding protein, it is suggested that bile acids may be the putative ligands.

Biological context

Fatty acid binding proteins (FABP) belong to a large family and bind, with variable stoichiometry, a diverse set of hydrophobic ligands, such as fatty acids, retinoids and bile salts, into a large cavity located in the interior of the protein. The specific function of these proteins still has to be determined, but it is clear that they are involved in fatty acid intracellular metabolism. Fatty acids are converted, within the cell, to many biological active compounds including oxygenated and hydroxylated metabolites, which play an important role in cell proliferation (Bratt, 2000).

More than sixty different FABP amino-acid sequences have been determined and generally classified on the basis of the organ from which they were initially isolated. The highest sequence similarity (up to 70–80%) is observed for FABPs from the same tissue from different species, sometimes quite distant in evolution, thus suggesting that tissue specificity could be correlated to protein function. However several examples are known in which more than one FABP type has been shown to be produced by a single tissue, with the proteins exhibiting variability both in the type, binding mode and stoichiometry of its preferred ligands. Along this line, the structural characterisation and binding properties of different FABPs purified from the same tissue is of relevance for the understanding of the molecular properties and function of this family.

Liver FABPs (L-FABP) identify a widely studied FABP sub-family (Börchers and Spener, 1993). Besides mammalian FABPs (human, mouse, cow, pig) sharing 79–90% sequence identity, a new basic protein (pI = 9), showing a very low sequence similarity with the mammalian species, was purified from chicken liver (Scapin et al., 1988) and hence called liver basic FABP (Lb-FABP). Later, other basic-type FABPs, exhibiting high sequence similarity, were isolated from the liver of other species, such as axolotl, catfish, lungfish, shark, frog, iguana (Di Pietro et al., 1999), though their structures are still unknown.

Here we report the first NMR structure of a basic-type FABP, the chicken Lb-FABP, and suggest, on the basis of comparison with the structures of other proteins of the family, its binding specificity. The study of the subtle structural changes brought about by evolution within the same protein family is relevant for the understanding of the sequence-function specificity relationships.

Methods and results

Chicken Lb-FABP (125 a.a.) was purified from natural sources (Scapin et al., 1988). The structure determina-

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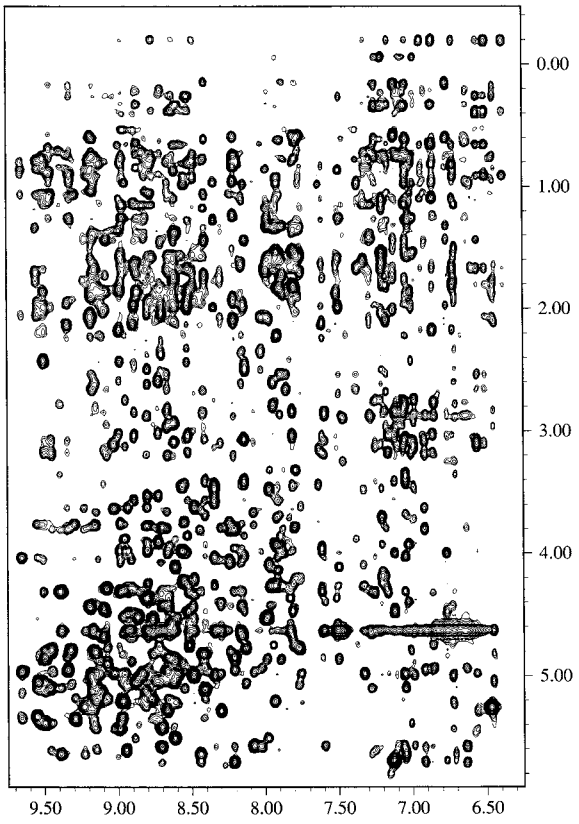

Figure 1. NH-aliphatic region of the 500 MHz ^{1}H NOESY spectrum of chicken Lb-FABP (1 mM in 20 mM phosphate buffer, pH = 5.6, 37 °C).

tion was performed at pH 5.6 and 37 °C. Homonuclear ^{1}H NMR experiments were recorded at 500 MHz on a Bruker DMX spectrometer. Spectra were analysed with the program XEASY (Bartels et al., 1995), while the programs DYANA (Güntert et al., 1997) and DISCOVER (Molecular Simulations, San Diego, CA, U.S.A.) were used for structure calculation and refinement, respectively.

The quality of the data can be estimated by the NH-aliphatic protons correlation region of the NOESY spectrum reported in Figure 1. The complete sequence-specific ^{1}H resonance assignment was obtained from standard ^{1}H 2D experiments and deposited in the BioMagResBank (accession code BMRB-5512). A partial assignment of this protein was previously reported (Schievano et al., 1999).

The large number of strong $d_{\alpha N}$ and $d_{\alpha\alpha}$ NOE connectivities indicated a high content of antiparallel beta-sheet in the protein. A survey of sequential short and medium range NOEs is reported in Figure 2. The reported secondary structures were also clearly consistent with the H^{α} and H^{N} shifts (data not shown). The ten antiparallel β-strands, β_A (3–12), β_B (37–43), β_C (46–53), β_D (56–63), β_E (67–72), β_F (76–85), β_G (88–93), β_H (96–103), β_I (106–113) and β_J (116–125) are connected by short type II turns except for β_A and β_B, which are connected by two short helical segments, α1 (14–21) and α2 (26–32). The two helices are antiparallel to each other, as demonstrated by the presence of NOEs between residues 14, 15, 18, 19 (α1 helix) and 28, 31, 32 (α2 helix). A β-bulge is present at positions 9–10 of β_A. A gap is observed between β_D and β_E: Only seven interstrand side-chain/side-chain NOEs have been identified between these two strands, thus limiting the size of the gap. This feature is common to all the FABPs structures determined to date.

Resonance signals of backbone amide protons of Lb-FABP showed partial protection against hydrogen/deuterium (H/D) exchange. After 14 h at 37 °C and pH 5.6 only 29 amides were left in the TOCSY spectrum, namely amides belonging to the following residues: 6–7 and 9 (β_A), 38–41 (β_B), 46–49 and 52 (β_C), 88–90 (β_G), 101 (β_H), 107–113 (β_I), 118–122 and 125 (β_J). Amides belonging to β_D, β_E, β_F strands and to the two helices exhibited very low protection.

A total of 1705 NOESY cross-peaks obtained with a mixing time of 100 ms were assigned and integrated, yielding 1179 non redundant constraints (345 intraresidue, 319 short, 135 medium and 380 long range contacts). Additional constraints were derived from measurements of 64 $^3J_{\alpha N}$ coupling constants.

Hydrogen bond formation or solvent exclusion from the amide protons was assumed to account for the slow and medium amide exchange rates. The partners for the detected hydrogen bonds were assigned on the basis of preliminary structures obtained by imposing only NOE restraints. The detected 25 hydrogen bonds were enforced by 50 distance restraints.

The statistics of the twenty conformers which resulted from a final DYANA calculation with 100 randomised starting conformations are given in Table 1. Each structure was minimised and the ten structures with the minimum potential energy and lowest number of violations were selected for further analysis (see Table 1). All the structures were analysed with PROCHECK-NMR (Laskowski et al., 1993). For the 10 best Lb-FABP conformers, a root-mean-square deviation (RMSD) value of 1.24 ± 0.14 Å was determined (Figures 3A and 3B). The overall fold of Lb-FABP, common to all known FABPs, consists of ten antiparallel β-strands (β_A–β_J) organised in two

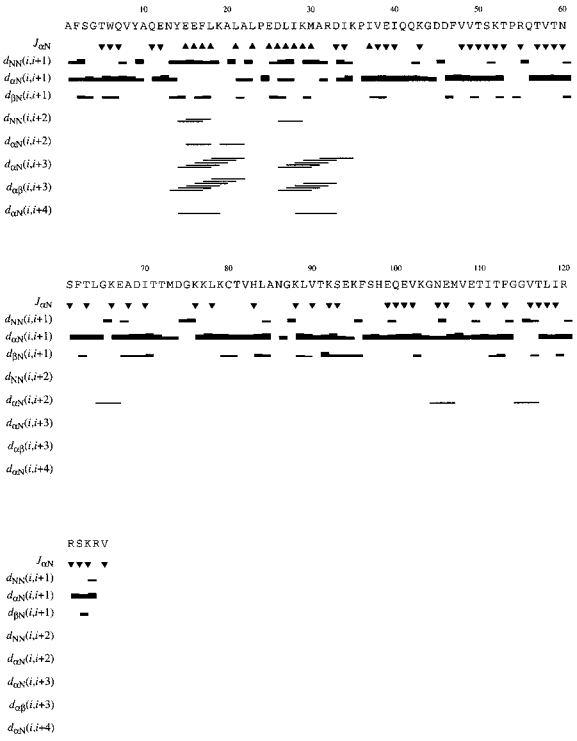

Figure 2. Amino acid sequence of chicken Lb-FABP and a survey of the observed short- and medium-range NOE connectivities. For the sequential NOEs $d_{\alpha N}$, d_{NN} and $d_{\beta N}$, thick and thin bars indicate strong and weak intensities. Medium range NOEs are indicated by lines connecting the two related residues. Up and down triangle identifies residues with $J_{\alpha N} < 5.5$ Hz and $J_{\alpha N} > 7.3$ Hz, respectively.

nearly ortogonal β-sheets that form a β-clam type structure with a gap between β_D and β_E strands. The two helices α1 and α2, inserted between β_A and β_B, close the protein cavity where small hydrophobic ligands, such as long chain saturated and unsaturated fatty acids, can be bound. The atom coordinates have been deposited in the Protein data bank (PDB ID code 1MVG). It is clear from the ensemble of NMR structures that certain regions display a greater backbone RMSD deviation, either because of the lack of structurally relevant NOEs or of a greater flexibility of these regions or both. The less defined regions comprise residues 21–24 (α1–α2 loop), 54–56 (β_C–β_D loop), 71–77 (β_E–β_F segment) and 91–95 (β_G–β_H segment). All these residues are located at the level of the so-called 'portal area' at the entrance of the internal cavity. H/D exchange data are consistent with a higher flexibility in the mentioned regions.

Two distinct hydrophobic clusters were identified, on the basis of NOE data. Cluster I, at the bottom

Table 1. Structure statistics of chicken Lb-FABP NMR final structures[a]

DYANA target function (Å^2)[b]	1.10 ± 0.31 (0.57...1.86)
NOE violations > 0.2 Å	1.3 ± 1.3 (0...4)
AMBER Energy (Kcal mol^{-1})	−533 ± 14 (−567...−508)
RMSD, N, C$^\alpha$, C′ (Å)[c]	1.24 ± 0.14 (0.95...1.49)
RMSD, N, C$^\alpha$, C′ (Å)[d]	1.00 ± 0.12 (0.66...1.29)
RMSD all heavy atoms (Å)[c]	2.06 ± 0.16 (1.60...2.36)

[a]Average values ± standard deviations for the final structures, minimum and maximum values for individual conformers in parentheses.
[b]Analysis of the best 20 DYANA structures before energy minimization.
[c]RMSD values for residues 3–125.
[d]RMSD values for residues 3–20, 25–53, 58–70, 78–90, 96–125.

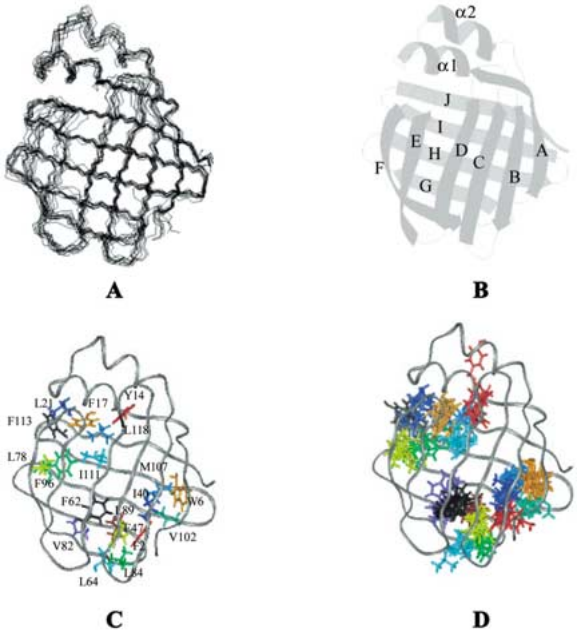

Figure 3. Superposition of the final ten best chicken Lb-FABP structures (A); a ribbon drawing of the structure with the lowest energy (B); in colour are the hydrophobic side-chains of residues belonging to cluster I and II (C); superposition of apo-FABPs residues corresponding to residues belonging to chicken Lb-FABP cluster I (D). The superimposed structures are: 1eal, 3ifb, 1ael, 1bwy, 1g5w.

of the hydrophobic cavity, included residues F2 (N terminus), W6 (β_A), I40 (β_B), F47 (β_C), F62 (β_D), L64 (CD loop), V82 and L84 (β_F), L89 (β_G), V102 (β_H) and M107 (β_I). Cluster II, at the opening of the calyx between the two helices and the barrel, included residues Y14 and F17 (α1 helix), L21 (α1–α2 loop), L78 (β_F), F96 (β_H), I111 and F113 (β_I) and L118 (β_J) (see Figure 3C).

Discussion and conclusions

A multiple sequence alignment obtained with ClustalW (http://www.ebi.ac.uk/clustalw/) using all the known sequences of basic-type and non basic FABPs indicated that the non basic protein with the highest similarity is pig ileal lipid binding protein (ILBP) (43% identity, 58% similarity). The structural comparison of Lb-FABP with all apo-FABPs of known structure (ileal binding protein, human and rat intestinal, bovine and human heart FABP) (Zhang et al., 1997; Hodson and Cistola, 1997; Lassen et al., 1995; Lücke et al., 1996, 2001), performed through the VAST search service (http://www.ncbi.nlm.nih.gov/Structure/VAST), indicated that ILBP has the highest structural similarity, exhibiting an RMSD for alignable regions of 1.61 Å. The RMSD with other apo-FABPs was higher and comprised between 2.02 and 3.16 Å.

The detected flexible segments of Lb-FABP are located at the portal area and, interestingly, correspond closely to the regions of ILBP involved in bile salts binding (Lücke et al., 1996). This observation suggests that Lb-FABP flexible regions have a functional role related to binding and that bile acids might be Lb-FABP putative ligands. In this line it is worth to stress that the residues important for bile acid binding in ILBP (W49, K77, F79, Y97, H99 and E110) (Lücke et al., 2000) are mostly conserved in Lb-FAB (V49, K76, L78, F96, H98 and E109) but not in chicken L-FABP (I49, E76, A78, L97, S99 and H110). In addition Lb-FABP, together with ILBP, displays a relatively low fatty acid binding affinity (K_d in the micromolar range) differently from other liver and liver basic proteins displaying much higher affinities for fatty acids (K_d in the nanomolar range) (Beringhelli et al., 2001; Lücke et al., 1996). These observations further support that fatty acids are not the primary ligands for this protein and that chicken liver and chicken liver basic proteins have different functional roles.

Cluster I, located at the bottom of the internal cavity, appears well conserved in the FABP family, independently from sequence similarity (Figure 3D). When hydrophobic residues of cluster I were superimposed to residues forming the internal hydrophobic cluster in bovine β-Lactoglobulin (BLG) (Ragona et al., 1997), a lipocalin belonging, together with FABPs, to the calycin structural superfamily, it was observed that seven out of eleven residues exhibited a similar orientation in the two proteins, thus indicating that this well conserved cluster is relevant to the calycins stability.

Cluster II involving residues belonging to C-terminal strands and to α1 and α2 helices, located at the entrance of the internal cavity, is much less conserved among different FABPs and may play some role in the binding specificity observed for different FABPs. Indeed a different mode of binding and a different stoichiometry was observed for FABPs from different tissues, with Lb-FABP exhibiting a different stoichiometry and a different binding mode with respect to L-FABP (Beringhelli et al., 2001). Further NMR investigations on the recombinant apo and holo Lb-FABP are now in progress to clarify mode, dynamics and specificity of binding.

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